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Physiological Response and Haematological Profile of Reproductive Ewe Consuming Diet Supplemented with Black Tea Extract and Sunflower Seed Oil

Didid Diapari*, Widya Hermana, Febrina Prameswari, and Anuraga Jayanegara

Department of Nutrition and Feed Technology, Faculty of Animal Science,
Bogor Agricultural University, Jl. Agatis Kampus IPB Darmaga, Bogor 16680
*** Corresponding author e-mail: didid.diapari1962@gmail.com**

Abstract. This study was aimed to examine the effect of black tea extract addition in diet containing 4% and 6% sunflower oil on ewe physiological responses and blood profiles from late pregnancy until early lactation. This study was designed using a 2×2 factorial completely randomized design with five replications on 20 late-pregnant ewes. The first factor was two levels of sunflower seed oil (4% and 6%) and the second factor was the levels of black tea extract addition (0 ppm and 500 ppm). Data were analyzed by using analysis of variance (ANOVA) and Duncan's multiple range test. Results showed that supplementation of sunflower seed oil and black tea extract had no effect on physiological responses and blood profiles of ewe during late pregnancy until early lactation except for erythrocyte numbers. Erythrocyte numbers were higher on 6% sunflower seed oil supplementation than that of 4% ($P<0.05$). In conclusion, the addition of black tea extract in diets containing 4% and 6% sunflower seed oil limitedly affect physiological responses and blood profiles of the ewe.

Key words: black tea extract, blood profile, ewe, physiological response, sunflower seed oil

Abstrak. Penelitian ini bertujuan untuk menguji pengaruh ekstrak teh hitam pada konsentrasi 500 ppm dalam ransum yang mengandung 4% dan 6% minyak biji bunga matahari terhadap respon fisiologis dan profil darah domba fase reproduksi akhir kebuntingan hingga awal laktasi. Desain penelitian menggunakan rancangan acak lengkap pola faktorial 2 × 2 dengan faktor pertama terdiri dari 2 level minyak biji bunga matahari (4% dan 6%) dan faktor kedua terdiri dari 2 level ekstrak teh hitam (0 ppm dan 500 ppm) dengan 5 ulangan. Domba yang digunakan adalah domba periode akhir kebuntingan sebanyak 20 ekor (bobot badan awal 31.2 ± 2.28 kg). Peubah yang diamati meliputi respon fisiologis dan profil darah. Data diolah menggunakan analisis ragam dan uji jarak berganda Duncan. Hasil menunjukkan bahwa suplementasi minyak biji bunga matahari dan ekstrak teh hitam tidak memberikan pengaruh nyata terhadap respon fisiologis dan profil darah domba kecuali untuk jumlah eritrosit. Pemberian 6% minyak biji bunga matahari secara nyata meningkatkan jumlah eritrosit dibandingkan dengan pemberian 4% minyak ($P<0.05$). Dapat disimpulkan bahwa penambahan 500 ppm ekstrak teh hitam dalam ransum mengandung 4% dan 6% minyak biji bunga matahari tidak mempengaruhi respon fisiologis dan profil darah domba.

Kata kunci*:* ekstrak teh hitam, domba, minyak biji bunga matahari, profil darah, respon fisiologis

Introduction

Indonesian local sheep have been known to have a genetic potential in the reproductive ability. Reproductive performance of the genetic factors will be expressed only if it is supported by conducive environmental factors such as the diet based on the nutrition requirement for reproductive ewe (Capper *et al*., 2006). Scaramuzzi *et al*. (2006) described that the reproductive performance is affected by quality and quantity of nutrients provided to pregnant ewes. Animals generally have four critical reproductive phases, i.e., during pre-mating, early pregnancy, the final third of gestation, and early lactation; these phases require nutritious diet in order to support fetus growth and development. Pregnant ewe requires a high concentration of nutrients in the diet at similar quantity because consumption will be reduced due to the reduction of stomach volume as a result of the rapidly growing fetus (Munoz-Gutierrez *et al.* 2002).

Sunflower seed oil contains high fat, and it is expected to sufficiently supply the energy

demand of ewe. Fat contains higher energy than that of carbohydrate or protein and produces lower heat increment. Low heat increment diet is apparently suitable for a tropical animal in order not to produce excessive heat. Thermoregulation process of continuous heat production is followed by heat release to the environment through skin and breath. This could be reflected in the measurement of physiological response parameters such as rectal temperature, respiration rate, heart rate, hematocrit, and heterophile to lymphocyte ratio.

Sunflower seed oil contains essential fatty acids such as linoleic acid 67.5% and oleic acid 18.7%. Diet containing unsaturated fatty acids could improve the fertility of ruminants (Cerri *et al*., 2009) but it is prone to oxidation and thus should be accompanied by antioxidants for protecting them from oxidation (Fremont *et al*., 1999). Antioxidant is defined as a compound that could prevent oxidative reactions of lipids from free radicals (Damayanthi *et al.,* 2004). Antioxidant activity in tea results from polyphenol (Gardner *et al.,* 2007). Black tea contains natural antioxidants, i.e. polyphenol (119.0-178.8 mg g^{-1}) and theaflavin (9.7-13.7 mg g^{-1}) to prevent peroxidation of unsaturated fatty acids in cell membrane phospholipids (Bhuyan *et al*., 2013).

The aim of this study was to examine the effect of black tea extract addition in diet containing 4% and 6% sunflower seed oil on the physiological responses of late pregnancy to early lactation ewe.

Materials and Methods

Animal and experimental diet

Twenty local sheep in late pregnancy to early lactation with an average body weight of 31.2 ± 2.28 kg were used in this study. The sheep were randomly distributed into individual cages. Maintenance of ewe was performed since late pregnancy until three weeks after giving birth. Temperature and humidity in the animal house were measured three times a day at 06.30 am, 12:30 pm, and 17:30 pm. Total mixed ration of grass and concentrate (30:70) was used as the experimental diet. The grass used in this study was *Brachiara humidicola*. The concentrate was composed of cassava, coconut meal, soybean meal, premix, salt, $CaCO_3$, sunflower seed oil and black tea extract. Extraction of black tea was conducted through maceration process of black tea powder dissolved in 70% ethanol with the ratio of ethanol, and black tea powder was 2:1. The mixture was allowed to stand for 24 hours and stirred every eight hours. The mixture was then filtered using filter paper and the filtrate was evaporated to obtain black tea extract. Black tea extract was mixed with tapioca flour with a proportion between tea extract and starch was 1:3.

This study employed a 2 x 2 factorial design based on a completely randomized design with 5 replications. First factor was 2 levels of sunflower seed oil supplementation (4% and 6%) and the second was 2 levels of black tea extract addition (0 ppm and 500 ppm). The treatment combinations were: M4T0 (0 ppm black tea extract + 4% sunflower seed oil), M4T500 (500 ppm black tea extract + 4% sunflower seed oil), M6T0 (500 ppm black tea extract + 6% sunflower seed oil), M6T500 (500 ppm black tea extract + 6% sunflower seed oil). Nutrient content of the experimental diets is presented in Table 1.

Table 1. Nutrient content of experimental diet (%DM)

Nutrient	Diet			
	M4T0	M4T500	M6T0	M6T500
DM (%)	64.5	68.4	69.2	69.2
Ash (%)	9.0	9.2	9.3	7.9
CP (%)	15.2	14.8	15.1	15.4
EE (%)	8.1	7.9	10.4	10.6
CF (%)	19.3	18.7	18.6	18.5
NFE (%)	48.4	49.4	46.6	47.6
TDN (%)	71.5	71.9	72.6	74.1

M4T0: 4% oil + 0 ppm black tea extract, M4T500: 4% oil + 500 ppm black tea extract, M6T0: 6% oil + 0 ppm black tea extract, M6T500: 6% oil + 500 ppm black tea extract.
DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, TDN: total digestible nutrient.

Physiological response

Parameters measured on physiological responses were respiration rate, heart rate and rectal temperature. Duplo measurement of respiration rate, heart rate, and rectal temperature were determined every week during the experimental period from late pregnancy until early lactation. The respiration rate was measured by placing the palms on the nose and count the breaths for 1 minute using a stopwatch and counter. Heart rate was measured by placing a stethoscope on the left chest while ewe was in standing position and count rate for 1 minute. Rectal temperature was measured by inserting a digital body thermometer into the rectum.

Haematological profile

Blood sampling was performed at the end of pregnancy period. An amount of 3 ml blood was drawn from the jugular vein of ewe using a sterile syringe, inserted into the tube with EDTA, and then taken to the laboratory for analysis. For hemoglobin determination, sahli tube was filled with HCl 0.1 N, then filled with 0.1 mL of blood until brown hematin acid formed. Distilled water was added until the color looked as the standard color. Blood level in the tube indicated the amount of hemoglobin in 100 mL/g blood. Hematocrit determination was performed by the microhematocrit method. Microcapiler pipe was filled with blood (about 4/5 part of the pipe volume) and then closed with stopper and centrifuged for 5 minutes. Hematocrit value was presented as erythrocyte volume percentage (%) read with microhematocrit reader.

For measuring number of red blood cells (erythrocytes) and white blood cells (leukocytes), blood sample was smoked using an erythrocyte pipette for red blood cells and leukocyte pipette for white blood cells, diluted with Hayem solution to the mark 101 for erythrocytes and diluted with Turk solution up to the mark 11 for leukocytes, and then homogenized. A drop of blood solution was dripped into the counting chamber covered with cover glass then observed under a microscope. Number of erythrocytes was calculated by looking at 25 boxes and observe the top corner right box, top left corner, middle, bottom right corner, and bottom left corner. Erythrocyte per mm^3 was calculated by summing erythrocytes that counted in 5 small boxes and multiplied by 10^4. Number of leukocytes was counted in 16 small boxes and observed 4 boxes on the top right corner, top left corner, bottom right corner, and bottom left corner. Leukocyte that counted in 16 squares was multiplied by 50. Leukocyte differentiation was calculated on 100 × 10 microscope magnification. Leukocyte

differentiation was made with 2 object glasses with the dropped blood then dried in the air, submerged in methanol solution for 5 minutes, and submerged in Giemsa solution for 30 minutes then washed. The number of lymphocytes, neutrophils, monocytes, and eosinophils counted in a zigzag up to a total of 100 items under the microscope. The percentage of each leukocyte derived from the number of each leukocyte differentiation was divided by the total number of leukocytes then multiplied by 100%.

Data analysis

Data were processed using analysis of variance (ANOVA) and the continued with Duncan's multiple range test when the ANOVA result showed significance at $P<0.05$. Statistical analysis was performed by employing SPSS software version 20.

Results and Discussion

Environmental condition

Research location had fluctuation temperature and humidity from morning until evening. Ambient temperature observed in the morning, day and evening ranged between 24.1-31.0°C with the humidity of 70-89% (Table 2). Comfort zone of tropical sheep typically ranges between 22-31°C (Subronto, 2003). Apparently the average temperature in the animal house was in the range of comfort zone and therefore suitable for ewe maintenance. Meanwhile, optimum humidity for sheep is less than 75% (Subronto, 2003). The average of the highest humidity in the morning (89%) exceeded the optimum humidity for sheep. This can be caused by a lack of air circulation inside the animal house so that water vapor is accumulated. A very high humidity is harmful since it may lead to slow and limited evaporation of water and potentially disturb animal thermoregulation system (Yani and Purwanto, 2006).

Table 2. Temperature and humidity during the experimental period

Time	Temperature (^oC)	Humidity (%)
Morning	24 ± 1.26	89 ± 4.33
Noon	31 ± 1.51	72 ± 8.99
Evening	30 ± 1.78	71 ± 8.32
Average	28.3 ± 0.26	77.3 ± 2.51

Physiological response

Neither sunflower seed oil nor black tea extract affected the respiratory rate of late pregnancy to early lactation ewe (Table 3). Respiratory rate during the late period of pregnancy to early lactation ewe was higher than the normal range of ewe in the tropics according to Suprayogi and Astuti (2006). Respiratory rate in late pregnancy period was different from the early lactation period. Ewe during late pregnancy period does not consume diet as much as during lactating period due to the limited capacity of digestive organs with the fetus inside. Lactating animals consume the highest dry matter intake among other reproductive phases to produce milk and energy reserves for preparing the next breeding season. Under such condition, higher oxygen intake is needed to run body metabolism during lactation period than during pregnancy period. Increased respiratory rate reflected heat quickly passed through blood to the body in order to help animals released body heat for balanced body temperature.

Supplementation of sunflower seed oil and black tea extract did not affect the heart rate of late pregnancy to early lactation ewe (Table 4). The heart rate of ewe in late pregnancy period was within a normal range according to Subronto (2003), i.e., 70-90 times min^{-1}. Black tea extract containing polyphenol is allegedly capable of improving blood circulation in order to maintain normal heart function. Provision of oil in diet could reduce body heat production so that the animals could improve their thermoregulation ability with lower heart rate.

Heart rate of ewe during early lactation was above normal range, higher in comparison to late pregnancy period. It is apparently associated with the increase of respiration rate during early lactation as compared with late pregnancy. An increase of respiration frequency may lead to an increase of heart rate since respiration muscles need more supply of oxygen and nutrients, and thus the heart pumped the blood faster.

An Addition of black tea extract and sunflower seed oil had no effect on rectal temperature of ewe during late pregnancy until early lactation (Table 5). Rectal temperature of ewe during late pregnancy to early lactation is still within the normal range according to Subronto (2003), i.e. between 38-40^{o}C. It seems that heat exposure to animal is already happened for a long time so that animal could adapt in balancing body heat production with the release of body heat to the environment. The normal rectal temperature may also occur as result of feeding the fat from sunflower seed oil with lower heat increment than that of protein and carbohydrate so that the animals were not constrained in releasing body heat.

Table 3. Respiratory rate (times/min) of ewe fed with experimental diet

		M4	M6	Average
Late Pregnancy	T0	50 ± 4.17	54 ± 13.70	52 ± 8.93
	T500	51 ± 8.03	52 ± 14.11	51.5 ± 11.07
	Average	50.5 ± 6.1	53 ± 13.90	51.75 ± 10
Early Lactation	T0	65 ± 7.13	58 ± 5.40	61.5 ± 6.26
	T500	61 ± 12.28	61 ± 9.40	61 ± 10.84
	Average	63 ± 9.70	59.5 ± 7.4	61.25 ± 8.55

T0: addition of 0 ppm black tea extract, T500: addition of 500 ppm black tea extract, M4: addition of sunflower seed oil 4%, M6: addition of sunflower seed oil 6%.

Table 4. Heart rate (times/min) of ewe fed with experimental diet

		M4	M6	Average
Late Pregnancy	T0	84 ± 8.23	84 ± 6.36	84 ± 7.29
	T500	85 ± 11.65	82 ± 7.17	83.5 ± 9.41
	Average	84.5 ± 9.94	83 ± 6.76	83.75 ± 8.35
Early Lactation	T0	102 ± 5.11	104 ± 4.84	103 ± 4.97
	T500	101 ± 11.5	102 ± 6.25	101.5 ± 8.87
	Average	101.5 ± 8.30	103 ± 5.54	102.25 ± 6.92

T0: addition of 0 ppm black tea extract, T500: addition of 500 ppm black tea extract, M4: addition of sunflower seed oil 4%, M6: addition of sunflower seed oil 6%.

Table 5. Rectal temperature (^{o}C) of ewe fed with experimental diet

		M4	M6	Average
Late Pregnancy	T0	39.0 ± 0.21	38.9 ± 0.30	38.95 ± 0.25
	T500	38.9 ± 0.19	38.9 ± 0.14	38.9 ± 0.16
	Average	38.95 ± 0.20	38.9 ± 0.22	38.92 ± 0.20
Early Lactation	T0	38.8 ± 0.15	38.9 ± 0.33	38.85 ± 0.24
	T500	38.7 ± 0.23	38.8 ± 0.20	38.75 ± 0.21
	Average	38.75 ± 0.19	38.5 ± 0.26	38.80 ± 0.22

T0: addition of 0 ppm black tea extract, T500: addition of 500 ppm black tea extract, M4: addition of sunflower seed oil 4%, M6: addition of sunflower seed oil 6%.

Blood profile

Generally, addition of black tea extract and sunflower seed oil in diet did not affect blood of ewe except for number of erythrocytes (Table 6). Concentrate with 6% sunflower seed oil showed higher erythrocytes number compared with concentrate with 4% sunflower seed oil (P<0.05). This result suggested that higher amount of oil in diet could increase number of erythrocytes. Essential fatty acids present in oil are part of lymphoid tissue that maintain immune system function by forming prostaglandin. The function of prostaglandin hormone is to repair cell membrane, including the membrane of red blood cells and thus could increase the number of erythrocytes (Fritsche et al., 1992)

Table 6. Haematological profile of ewe fed with experimental diet

Parameter	Normal *		T0	T500	Average
Hematocrit (%)	27-45	M4	29.3 ± 1.52	31.7 ± 2.08	30.5 ± 1.80
		M6	30.3 ± 1.52	32.3 ± 2.51	31.3 ± 2.01
		Average	29.8 ± 1.52	32.0 ± 2.29	30.9 ± 1.90
Hemoglobin (g/dl)	9-15	M4	9.36 ± 1.18	9.46 ± 0.80	9.41 ± 0.99
		M6	8.75 ± 0.66	9.13 ± 0.23	8.94 ± 0.44
		Average	9.05 ± 0.92	9.29 ± 0.51	9.17 ± 0.71
Eryhtrocytes (million/mm^3)	9-15	M4	7.40 ± 0.61	8.61 ± 0.77	8.00 ± 0.69^a
		M6	10.04 ± 0.69	9.45 ± 1.11	9.74 ± 0.90^b
		Average	8.72 ± 0.65	9.03 ± 0.94	8.87 ± 0.79
Leucocytes (thousands/mm^3)	8-12	M4	8.01 ± 0.21	9.10 ± 1.65	8.55 ± 0.93
		M6	9.02 ± 0.45	10.25 ± 0.35	9.63 ± 0.40
		Average	8.51 ± 0.33	9.67 ± 1.00	9.09 ± 0.66
Leukocyte Differentiation					
Lymphocytes (%)	50-70	M4	57.7 ± 2.88	58.0 ± 4.00	57.8 ± 3.44
		M6	56.7 ± 3.21	58.0 ± 7.00	57.3 ± 5.10
		Average	57.2 ± 3.04	58.0 ± 5.50	57.6 ± 4.27
Neutrophils (%)	30-48	M4	37.0 ± 1.00	34.0 ± 4.35	35.5 ± 2.67
		M6	38.3 ± 2.08	33.3 ± 4.50	35.8 ± 3.29
		Average	37.7 ± 1.54	33.7 ± 4.42	35.7 ± 5.96
Basophils (%)	0-3	M4	1.33 ± 1.15	2.00 ± 0.01	1.66 ± 1.15
		M6	1.67 ± 1.15	3.00 ± 1.00	2.33 ± 1.07
		Average	1.50 ± 1.15	2.50 ± 0.50	1.99 ± 1.11
Monocytes (%)	0-4	M4	1.67 ± 1.15	2.67 ± 0.57	2.16 ± 1.09
		M6	2.00 ± 1.73	3.33 ± 0.57	2.66 ± 1.15
		Average	1.83 ± 1.44	3.00 ± 0.57	2.40 ± 1.00
Eusinophils (%)	1-8	M4	2.33 ± 0.57	3.33 ± 1.15	2.83 ± 0.86
		M6	1.33 ± 0.57	2.33 ± 1.52	1.83 ± 1.04
		Average	1.83 ± 0.57	2.83 ± 1.33	2.33 ± 0.95
Neutrophils /Lymphocytes	0.5	M4	0.64 ± 0.04	0.59 ± 0.11	0.61 ± 0.07
		M6	0.67 ± 0.07	0.58 ± 0.15	0.62 ± 0.11
		Average	0.65 ± 0.05	0.58 ± 0.13	0.61 ± 0.09

* Normal value by Schalm (2010).
T0: addition of 0 ppm black tea extract, T500: addition of 500 ppm black tea extract, M4: addition of sunflower seed oil 4%, M6: addition of sunflower seed oil 6%.
Different superscripts in the same column are significantly different at P<0.05.

Ewe hemoglobin value in this experiment was within the normal range according to Schalm (2010), apparently due to adequate Fe supply such as from sunflower seed oil that contains high Fe, i.e. 49.66 mg kg^{-1} (Özcan, 2006). Ewe hematocrit values were also within the normal range, comparable to the hemoglobin and red blood cell count reported by Mawati *et al*. (2004). This may reflect that ewe has been adapted under tropical condition. Number of leucocytes as well as leukocyte differentiation for all treatments were within the normal range, indicating that immune system was not compromised by feeding treatment.

Neutrophil to lymphocyte ratio is an indicator of animal response to environmental change (Maheswari, 2008). High ratio of neutrophil to lymphocyte indicates that animals experiencing stress (Kannan, 2000). Stress produces glucocorticoid hormones that may disturb immune cells. Glucocorticoid disturbed the cytokines production that necessary for immune responses (Mashaly *et al*., 2004). Stress may lead to leukocytosis that is caused by increasing neutrophil from glucocorticoids induced and release of neutrophil reserves in the marrow bone (Sugito and Delima, 2009). Standard value for neutrophil to lymphocyte ratio in sheep is 0.5 (Schalm, 2010). Data in the present study showed that ewe neutrophil to lymphocyte ratio were above normal for all treatments. This could be due to animal exposure to fluctuating temperature, causing stress of the animals.

Conclusions

Supplementation of black tea extract and sunflower seed oil did not affect physiological responses and majority of blood parameters of ewe during late pregnancy until early lactation. Supplementation of 6% sunflower seed oil in diet increased erythrocytes of ewe as compared with 4% sunflower seed oil.

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